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# A Genetic Analysis of Synergistic Action of Sulfonamide Derivatives with DDT against House Flies

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## 摘 要

物体の表面に残留する毒物に、害虫をばく露してこれを駆除する方法において、その表面に残留する薬量と、これにばく露する時間のふたつは、生死を決定する最大の要因と考えられるが、野外実験の規模においては、このふたつはさまざまな組合わせを生じ、昆虫の生死はこれらの総和によってきめられるはずである。Dieldrin のゴキブリに対する毒性をこの見地から検討するため、濾紙法によって生死を個体別に記録し、その結果をプロビット平面に表現する方法によって解析した。

塗布薬量、ばく露時間の対数を、それぞれ  $x_1, x_2$  としたとき、致死率のプロビット  $Y$  との関係は、 $Y = -6.6095 + 1.5090x_1 + 4.2689x_2$  の方程式をもって表現することができた。 $\chi^2$  試験の結果、実験値と理論値とは抽出誤差の範囲内で適合することが証明された。 $P=0.5$  および  $0.9$  の信頼限界を計算し図に示した。

## 引 用 文 献

- 1) Finney, D. J.: *Biometrika* 34, 320~34 (1947).

- 2) 長沢純夫・柴三千代: 防虫科学 29, 46~51 (1964).

## Summary

Residual toxicity of dieldrin to the German cockroach was evaluated from the individual records of dead or alive in the filter paper test. From the data of sixty-three combinations of residual dose per weight of roach  $D$  (mg/g) and time of exposure  $T$  (min) together with indication of whether or not the cockroach responded, an equation for fitting a probit regression plane was estimated as  $Y = -6.6095 + 1.5090x_1 + 4.2689x_2$ . Here,  $x_1$  and  $x_2$  are  $\log D$  and  $\log T$  respectively and  $Y$  is the probit probability of response  $P$ . Result of the  $\chi^2$  test showed no significant difference between the empirical probits and the predictions from the fitted equation. The 5% fiducial limits curves ( $t=1.96$ ) for  $P=0.5$  and  $0.9$  were calculated and shown in figures.

**A Genetic Analysis of Synergistic Action of Sulfonamide Derivatives with DDT against House Flies.** Zen-ichi OGITA and Tsutomu KASAI\* (Department of Genetics, Medical School, Osaka University, Osaka.) Received October 31, 1965. *Botyu-Kagaku*, 30, 119, 1965.

20. イエバエにおける DDT とスルホンアミド誘導体の共力作用機構の遺伝学的解析  
荻田 善一・笠井 勉\* (大阪大学医学部遺伝学教室) 40. 10. 31 受理

DDT 共力剤の一種である Sulfonamide 系の WARF Antiresistant の共力作用機構に関する解析をおこなった。Antiresistant は DDT に中程度の抵抗性を示す系統には DDT と混合することによって顕著な共力作用をもたらすが、非常に抵抗性の高い系統や感受性系統に対しては共力作用を示さなかった。この機構を明らかにするため DDT に対する抵抗性遺伝子との関係について遺伝学的解析をおこなった。イエバエの DDT 単独に対する抵抗性は主として第5染色体上の優性遺伝子と第2染色体上の劣性遺伝子によって支配されているが、DDT と Antiresistant の混合剤に対する抵抗性は第2染色体上の DDT 抵抗性をもたらす劣性遺伝子のみが関係することが明らかとなった。第5染色体上の DDT 抵抗性の遺伝子は DDT 脱塩酸酵素活性を支配していることが知られているので Antiresistant の共力作用はこの酵素を阻害することによってもたらされることが結論された。また Antiresistant と血糖降下剤である Sulfonyleurea 剤との化学構造の類似性から種々の Sulfonamide について DDT との共力作用とマウスに対する血糖降下作用との関係についても論議した。

Many investigators have reported on DDT-resistance in the house fly from a genetical or biochemical viewpoint. Sternburg *et al.*<sup>1)</sup> (1954) reported that various strains of the house fly which are resistant to DDT, contain an amount of DDT-dehydrochlorinase corresponding to the level of

resistance of the strain to DDT. DMC, an analogue of DDT, acts as an inhibitor of DDT-dehydrochlorinase (Abedi *et al.*<sup>2)</sup> 1963), so that it is a synergist for DDT against DDT-resistant insects. Inheritance of DDT-resistance is controlled by at least two factors in the house fly (Tsukamoto and

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Suzuki<sup>3)</sup> 1964). A sulfonamide compound N-di-*n*-butyl-*p*-chlorobenzenesulfonamide, available under the name of WARF Antiresistant, has proved to be a highly effective DDT synergist against DDT-resistant insects.<sup>4,5)</sup>

The present investigation was intended to clarify relations between chemical structure and synergistic action of sulfonamide derivatives with DDT, and to analyze the synergistic action of the Antiresistant on DDT-resistant house flies from a genetic basis.

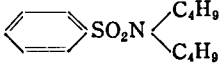
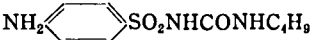
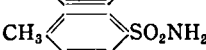
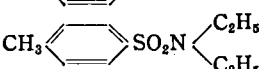
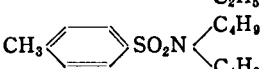
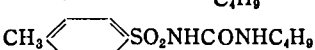
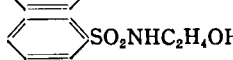
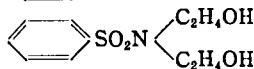
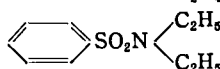
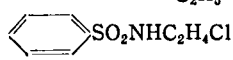
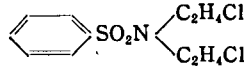
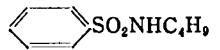
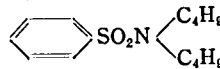
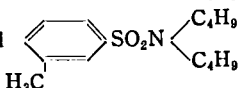
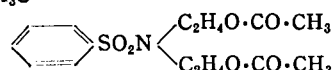
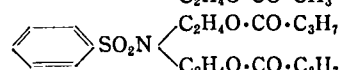
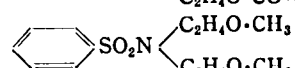
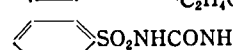
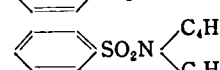
### Materials and Methods

Insecticide-resistant and susceptible strains of the house fly, *Musca domestica*, kept in our laboratory were used in this study. Their insecticide susceptibility, origins and rearing methods have been described in detail (Ogita and Kasai<sup>6)</sup> 1965). The *JIR* strain was established as DDT-resistant strain under DDT pressure by Dr. Tsukamoto. Synergistic action against house flies was estimated by the topical application. One  $\mu$ l of acetone solution containing DDT with or without sulfonamide was applied to dorsum of the thorax of ether anesthetized house flies by a microsyringe. The treated flies were kept at 25°C and their mortality was counted 24 hours later. Usually, 50 females and 50 males were used as a group for each determination. Another method of estimation of insecticidal activity was performed with a contact method. One ml of acetone solution of insecticides was pipetted on a sheet of filter paper which was confined in a petri dish. One hour later, after evaporation of the solvent, ten house flies were introduced into the dish, then knock down counts were performed at appropriate intervals. For the determination of linkage groups of DDT-resistance and resistance to the mixture consisting of DDT and Antiresistant, 1 day old  $F_2$ -progeny flies from a backcross {*ro*; *ct* ; *cm* ♀ ×  $F_1$  (*ro* ; *ct* ; *cm* ♀ × *JIR* ♂) ♂} or  $F_2$  flies of a cross (*ro* ; *ct* ; *cm* ♀ × *JIR* ♂) were treated with DDT or the mixture by topical application, and mortality counts were performed on each phenotype. Statistical analysis of dominant and recessive resistance factors was made by the method suggested by Tsukamoto<sup>7)</sup> (1964).

The sulfonamide derivatives used in this study and *p*, *p'*-DDT was kindly provided by Messrs. T. Ohno, and I. Takeda of Chemical Institute,

Japan Agricultural Chemicals and Insecticides Co., Ltd.

Table 1. Synergistic action of sulfonylamides for DDT against the *Takatsuki* strain of house flies.

No.	Chemical*	Mortality with DDT**
1		42
2		38
3		28
4		50
5		38
6		60
7		46
8		30
9		100
10		100
11		100
12		100
13		100
14		98
15		38
16		22
17		46
18		90
19		100

\* One  $\mu$ l of acetone solution containing 10mM DDT with 10mM of each chemical was treated to female house flies by the topical application.

\*\* The treatment of DDT alone brought about the mortality ranging 22~42%.

## Results

### 1. Chemical structures and synergistic activity.

In order to clarify the relation between chemical structure and synergistic activity of sulfonamides with DDT, many sulfonamide-derivatives were tested for their synergistic action (Table 1). Variations in the aryl portion of the molecule brought about a marked effect on its synergistic activity. Unsubstituted phenyl derivatives were less active; *p*-substituent with  $-\text{NH}_2$  radical was also less active, whereas those with  $-\text{CH}_3$  radical were moderately active. The most effective of all *p*-substituents studied was halogen (Cl, Br), and especially chlorine. Next, variations in the alkyl portion of the *p*-chlorobenzenesulfonamide were studied. Compounds in which N-atom was mono- or di-substituted with  $-\text{C}_2\text{H}_5$ ,  $-\text{C}_2\text{H}_4\text{Cl}$ ,  $-\text{C}_4\text{H}_9$  brought about the most effective synergistic action, whereas substitution with  $-\text{C}_2\text{H}_4\text{OH}$ ,  $-\text{C}_2\text{H}_4\text{OCO}\cdot\text{CH}_3$ ,  $-\text{C}_2\text{H}_4\text{OCH}_3$  resulted in very weak synergistic activity. Chlorpropamide, a well known hypoglycemic sulfonylurea (No. 18 in Table 1), proved to be moderately active synergist for DDT. Thus the most effective synergists of all the compounds studied were N-dialkyl *p*-chlorobenzenesulfonamides, such as, Antiresistant and its ethyl analogue.

### 2. Synergistic action to several strains.

Synergistic action of Antiresistant to DDT was examined in several strains of the house fly, both resistant and susceptible to DDT (Table 2).

Table 2. Synergistic action of WARF Antiresistant for DDT against several strains of the house fly.

Strain	LD-50 $\mu\text{g}$ per fly	
	DDT	DDT + Antiresistant
<i>ro; ct; cm</i>	0.12	0.15
<i>RP</i>	4	0.8
<i>Takatsuki</i>	8	0.5
<i>K-3926</i>	100<	100<
<i>JIR</i>	100<	100<

Antiresistant did not increase the insecticidal action of DDT against a susceptible strain, *ro; ct; cm*, whereas it markedly synergized against moderately DDT-resistant strains, *Takatsuki* or *PR*. However, no synergistic action was observed

with Antiresistant against highly DDT-resistant strains, such as *K-3926* and *JIR*. Treatment with 100 $\mu\text{g}$  DDT plus 100 $\mu\text{g}$  Antiresistant or 100 $\mu\text{g}$  DDT did not kill any of the flies of these two strains. The synergistic action of Antiresistant with DDT was tested against *Takatsuki* strain at various concentrations, to which the synergistic action was the most effective within the strains tested. This action was evaluated by the contact method and the experimental results were expressed as KT-50 values (Fig. 1), which were determined

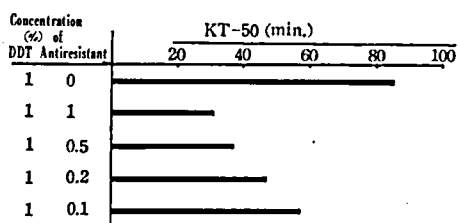


Fig. 1. Synergistic action of Antiresistant with DDT at various concentrations against *Takatsuki* strain of house flies. Based on 50 females for each concentration.

on the basis of 50 females for each concentration. The higher concentration brought about the more effective synergistic action. Thus, the mixture of 1% DDT with 1% Antiresistant was most effective. Synergistic action was also observed at 1:10 ratio of Antiresistant to DDT, though to a lesser degree.

### 3. Genetic analysis.

As the *JIR* strain was highly resistant both to DDT and to mixture of DDT and Antiresistant, resistant factors were analyzed on a genetic basis in order to clarify whether DDT resistance and mixture-resistance were controlled by the same mechanism or not. Fig. 2 shows the resistance levels of the susceptible *ro; ct; cm* strain,  $F_1$  hybrids, and

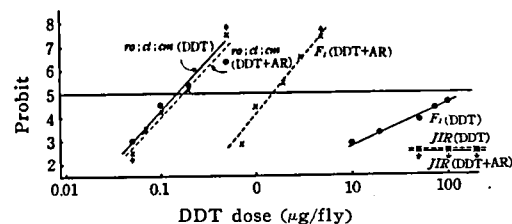


Fig. 2. Resistance levels of susceptible (*ro; ct; cm*), resistant (*JIR*) strains and the  $F_1$  hybrid to DDT and DDT + Antiresistant (1:1 ratio). AR = Antiresistant.

the resistant *JIR* strain both to DDT and to the mixture. The *JIR* strain was highly resistant not only to DDT but also to a high dose range of the mixture. However,  $F_1$  hybrids were not so resistant to the mixture as DDT. The  $F_1$  hybrids proved to be intermediate both in resistance to DDT and to the mixture, approaching the more resistant parent in the case of DDT, but approaching the more susceptible one when the mixture was employed. This suggested DDT-resistance was controlled by the incompletely dominant factor whereas mixture-resistance the incompletely recessive factor.

Linkage group of the resistance factors was studied by crossing experiments. Ten  $\mu$ g of DDT alone per fly was topically applied to  $F_2$  progeny obtained from a backcross ( $ro; ct; cm \text{ } \varphi \times F_1 (ro;$

$ct; cm \text{ } \varphi \times JIR \text{ } \delta) \text{ } \delta$ ), and number of survivals of each phenotype was counted 24 hours later (Table 3). This dose was chosen for analysis of dominant factor, for it does not kill the heterozygotes. The results indicated phenotypic *cm* progenies in which both 5th chromosomes were derived from susceptible strain were more susceptible than 5th chromosomal wild progenies which had the 5th chromosome carrying a factor of resistance derived from the *JIR* strain in heterozygous condition. These data evidently indicate that the most important effect on DDT-resistance may be due to the 5th chromosome. The 2nd chromosomal effect is also marked. Results of statistical analysis of the data (Table 4) also indicate that DDT-resistance is controlled by multifactorial genetic system in which both 5th and 2nd chromosomal

Table 3. Survival rate of each phenotypic  $F_2$ -progeny obtained from a backcross ( $ro; ct; cm \text{ } \varphi \times F_1 (ro; ct; cm \text{ } \varphi \times JIR \text{ } \delta) \text{ } \delta$ ) following topical application of 10 $\mu$ g DDT per fly.

Phenotype	Female			Male			Pooled 0	Mean 0
	No. of flies	Survival %	Arcsin 0	No. of flies	Survival %	Arcsin 0		
+ ; + ; +	70	94.29	76.19	75	89.33	70.91	147.10	73.55
<i>ro</i> ; + ; +	50	34.00	35.67	52	32.69	34.88	70.55	35.28
+ ; <i>ct</i> ; +	38	89.47	71.09	46	95.65	78.03	149.12	74.56
<i>ro</i> ; <i>ct</i> ; +	51	11.76	20.09	49	14.29	22.22	42.31	21.16
+ ; + ; <i>cm</i>	50	4.00	11.54	51	13.73	21.72	33.26	16.63
<i>ro</i> ; + ; <i>cm</i>	26	0	0	34	8.82	17.26	17.26	8.63
+ ; <i>ct</i> ; <i>cm</i>	40	15.00	22.79	49	16.33	23.81	46.60	23.30
<i>ro</i> ; <i>ct</i> ; <i>cm</i>	32	0	0	48	0	0	0	0

Table 4. Statistical analysis for dominant effect of chromosomes on DDT-resistance (from Table 3).

Chromosome	Resistance effect	Sum of squares	Degree of freedom	Mean square	F
(Total)	—	11335.71	15	—	—
(Phenotypes)	253.11	11091.27	7	1584.47	60.67**
2	122.97	3782.25	1	3782.25	144.82**
4	15.07	56.79	1	56.79	2.17
2 $\times$ 4	-30.43	231.45	1	231.45	8.86*
5	155.99	6084.00	1	6084.00	232.95**
2 $\times$ 5	60.33	909.93	1	909.93	34.84**
4 $\times$ 5	11.15	31.08	1	31.08	1.19
2 $\times$ 4 $\times$ 5	0.17	0.01	1	0.01	0.00
(Sex)	—	61.62	1	61.62	2.36
(Error)	—	182.82	7	26.12	—

\* Significant at 5% level,

\*\* Significant at 1% level.

Table 5. Survival rate of each phenotypic  $F_2$ -progeny obtained from a backcross  $\{ro; ct; cm \text{♀} \times F_1 (ro; ct; cm \text{♀} \times JIR \text{♂}) \text{♂}\}$  following topical application of  $0.5 \mu\text{g}$  DDT+ $0.5 \mu\text{g}$  Antiresistant per fly.

Phenotype	Female			Male			Pooled $\theta$	Mean $\theta$
	No. of flies	Survival %	Arcsin $\theta$	No. of flies	Survival %	Arcsin $\theta$		
$+++$	92	56.52	48.73	94	70.21	56.91	105.64	52.82
$ro+++$	66	16.67	24.12	68	8.82	17.26	41.38	20.69
$+ct+$	77	31.17	33.96	76	42.11	40.46	74.42	37.21
$roct+$	21	14.29	22.22	35	5.71	13.81	36.03	18.02
$+++cm$	88	2.27	8.72	83	1.20	6.29	15.01	7.51
$ro+cm$	64	0	0	68	0	0	0	0
$+ctcm$	36	13.89	21.89	58	5.17	13.18	35.07	17.54
$roctcm$	44	2.27	8.72	52	0	0	8.72	4.36

Table 6. Statistical analysis for dominant effect of chromosomes on mixture-resistance (from Table 5).

Chromosome	Resistance effect	Sum of squares	Degree of freedom	Mean square	F
(Total)	—	4554.45	15	—	—
(Phenotypes)	158.15	4362.85	7	623.26	26.39**
2	72.01	1296.36	1	1296.36	54.88**
4	3.89	3.78	1	3.78	0.16
$2 \times 4$	7.27	13.21	1	13.21	0.56
5	99.33	2466.61	1	2466.61	104.43**
$2 \times 5$	30.63	234.55	1	234.55	9.93*
$4 \times 5$	32.67	266.83	1	266.83	11.30*
$2 \times 4 \times 5$	18.61	86.58	1	86.58	3.67
(Sex)	—	26.27	1	26.27	1.11
(Error)	—	165.34	7	23.62	—

\* Significant at 5% level,

\*\* Significant at 1% level.

factors are major ones. Dominant factors of mixture-resistance were genetically analyzed at  $0.5 \mu\text{g}$  DDT+ $0.5 \mu\text{g}$  Antiresistant per fly. These doses would not kill heterozygotes. The results (Tables 5 and 6) indicate both 2nd and 5th chromosomal factors are also responsible for mixture-resistance.

However, highly resistant factor to the mixture seems to be a recessive one (Fig. 2), so that  $F_2$  progeny flies of a cross  $(ro; ct; cm \text{♀} \times JIR \text{♂})$  were employed for analysis of factors of DDT- and mixture-resistance. Results of topical application of  $20 \mu\text{g}$  DDT per fly (dominant and recessive factors) indicated that both 2nd and 5th chromosomal factors are also major DDT-resistance factors (Tables 7 and 8). For the analysis of mixture-

resistance, doses of  $20 \mu\text{g}$  DDT+ $20 \mu\text{g}$  Antiresistant were employed, which might bring about the kill in susceptible and heterozygous flies, whereas they would not kill homozygous resistant ( $JIR$ ) flies. In the  $F_2$  progenies of this cross, theoretically two third of the wild type flies are heterozygous, and one third is homozygous as to each chromosome. Therefore, it was expected that only one fourth of the treated flies which were homozygous in the resistance gene could survive at this dosage. In the experiment, only low percentage survival was observed. As shown in Table 9, survival rates of  $+++$ ,  $+ct+$ ,  $+++cm$ , and  $+ctcm$  progenies, in which one or both of the 2nd chromosomes were derived from the resistant strain, were higher than  $ro+++$ ,  $roct+$ ,  $+$ ,

Table 7. Survival rate of each phenotypic  $F_2$ -progeny obtained from a cross ( $ro; ct; cm \text{♀}$   $\times JIR \text{♂}$ ) following topical application of  $20\mu\text{g}$  DDT per fly.

Phenotype	Female			Male			Pooled $\theta$	Mean $\theta$
	No. of flies	Survival %	Arcsin $\theta$	No. of flies	Survival %	Arcsin $\theta$		
$+++$	334	92.22	73.78	244	84.02	66.42	140.20	70.10
$ro+++$	65	63.08	52.59	46	45.65	42.53	95.12	47.56
$++ct+$	114	85.96	68.03	109	89.91	71.47	139.50	69.75
$ro; ct; +$	10	40.0	39.23	18	55.56	48.22	87.45	43.73
$+++cm$	82	39.02	38.65	72	36.11	36.93	75.58	37.79
$ro; ++cm$	20	0	0	11	0	0	0	0
$++ctcm$	26	26.92	31.24	28	35.71	36.69	67.93	33.97
$ro; ct; cm$	11	0	0	9	0	0	0	0

Table 8. Statistical analysis for combined (dominant and recessive) effect of chromosomes on DDT-resistance (from Table 7).

Chromosome	Resistant effect	Sum of squares	Degree of freedom	Mean square	F
(Total)	—	10272.46	15	—	—
(Phenotypes)	302.90	10132.11	7	1447.44	72.23**
2	120.32	3618.02	1	3618.02	180.54**
4	8.00	16.00	1	16.00	0.80
$2 \times 4$	0.34	0.03	1	0.03	0.00
5	159.38	6352.09	1	6352.09	316.97**
$2 \times 5$	-23.20	134.56	1	134.56	6.71*
$4 \times 5$	0.36	0.03	1	0.03	0.00
$2 \times 4 \times 5$	-7.28	13.25	1	13.25	0.66
(Sex)	—	0.09	1	0.09	0.00
(Error)	—	140.26	7	20.04	

\* Significant at 5% level,

\*\* Significant at 1% level.

Table 9. Survival rate of each phenotypic  $F_2$ -progeny obtained from a cross ( $ro; ct; cm \text{♀}$   $\times JIR \text{♂}$ ) following topical application of  $20\mu\text{g}$  DDT +  $20\mu\text{g}$  Antiresistant per fly.

Phenotype	Female			Male			Pooled $\theta$	Mean $\theta$
	No. of flies	Survival %	Arcsin $\theta$	No. of flies	Survival %	Arcsin $\theta$		
$+++$	405	24.44	29.60	426	26.29	30.85	60.45	30.23
$ro+++$	134	0.74	4.93	136	1.47	7.04	11.97	5.99
$++ct+$	88	21.59	27.69	71	18.31	25.33	53.02	26.51
$ro; ct; +$	40	0	0	41	0	0	0	0
$+++cm$	73	15.07	22.87	66	24.24	29.47	52.34	26.17
$ro; ++cm$	18	0	0	11	0	0	0	0
$++ctcm$	31	16.13	23.66	30	6.67	15.00	38.66	19.33
$ro; ct; cm$	14	0	0	10	0	0	0	0

Table 10. Statistical analysis for recessive effect of chromosomes on mixture-resistance (from Table 9).

Chromosome	Resistant effect	Sum of squares	Degree of freedom	Mean square	F
(Total)	—	2560.60	15	—	—
(Phenotypes)	108.23	2494.52	7	356.36	38.64**
2	96.25	2316.02	1	2316.02	251.16**
4	16.55	68.49	1	68.49	7.43*
2×4	4.57	5.22	1	5.22	0.57
5	17.23	74.22	1	74.22	7.86*
2×5	5.25	6.89	1	6.89	0.75
4×5	2.87	2.06	1	2.06	0.22
2×4×5	-9.11	20.75	1	20.75	2.25
(Sex)	—	1.53	1	1.53	0.17
(Error)	—	64.55	7	9.22	—

\* Significant at 5% level,

\*\* Significant at 1% level.

*ro*; +; *cm* and *ro*; *ct*; *cm* progenies which had both the 2nd chromosomes derived from the susceptible strain. These data evidently indicate that the most important effect on the mixture-resistance may be due to the 2nd chromosome. Results of factorial analysis of the data shown in Table 10 indicate more exactly that the 2nd chromosomal resistance factor is the major one, and that the effect of the 5th chromosomal DDT-resistance factor is considerably depressed by Antiresistant. As mentioned previously, many investigators showed that a close correlation exists between DDT-resistance and DDT-dehydrochlorinase activity. Antiresistant is a useful synergist for DDT to DDT-resistant flies which were brought about by increased DDT-dehydrochlorinase activity, owing to its ability to inhibit the enzyme activity. However, there are other mechanism of resistance than dehydrochlorination of DDT, as controlled by 2nd chromosomal factor of the house fly. Antiresistant can not act as a synergist for DDT against these DDT-resistant flies.

### Discussion

Many experiments have been performed on various biological activities of sulfonamides. Certain sulfonamides were found to have a strong bactericidal activity, which is particularly directed against streptococci.<sup>8)</sup> Sulfonamides gave effective control when applied either through the roots or leaves

against plant pathogens, including *Uromyces fabae* on the broad bean and *Puccinia tritricina* on the wheat (Crowdy *et al.*<sup>9)</sup> 1958). It was proposed that the disease control resulted from the systemic distribution of an active compound. Hypoglycemic action of sulfonamidederivatives has also been well established.<sup>10)</sup> The sulfonylurea compounds are now widely employed in the treatment of diabetes mellitus. A considerable literature has been accumulated on the pharmacological and clinical properties of sulfonylurea drugs.

Neeman *et al.*<sup>11)</sup> (1956) reported that a series of 4-bromobenzenesulfon-4'-chloroanilides increased the insecticidal action of DDT against strain (*M*) of house flies, moderately resistant to DDT. Butyl-antiresistant and its analogues were found to have effective synergistic activity for DDT against DDT-resistant flies. The present results on relations between chemical structure and synergistic action of sulfonamide derivatives indicated; (1) *p*-chlorobenzene structure is most important, and (2) butyl radical of alkyl portion bring about effective activity. McLamore *et al.*<sup>12)</sup> (1959) reported on the effects of structural changes on hypoglycemic activity of sulfonylurea drugs, and suggested that chlorpropamide (1-propyl 3-*p*-chlorobenzene sulfonylurea) is the most potent and longest acting of all the sulfonylureas studied in their program. Structural similarity between synergists for DDT and hypoglycemic agents led



Table 11. Hypoglycemic activity of sulfonamides by oral administration against mice.

No.	Chemical	Hypoglycemic activity					
		0	1	2	4	8	24(hrs.)
1	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_2\text{H}_4\text{OH} \\ \text{C}_2\text{H}_4\text{OH} \end{cases}$	100	133	115	108	86.0	127
2	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{NH}\cdot\text{C}_2\text{H}_4\text{Cl}$	100	148	90.3	103	87.2	97.1
3	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_2\text{H}_4\text{Cl} \\ \text{C}_2\text{H}_4\text{Cl} \end{cases}$	100	92.2	74.6	60.9	65.5	68.7
4	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_4\text{H}_9 \\ \text{C}_4\text{H}_9 \end{cases}$	100	112	83.6	51.7	41.4	54.5
5	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_4\text{H}_9 \\ \text{C}_4\text{H}_9 \end{cases}$ $\text{H}_3\text{C}$	100	105	102	77.7	73.1	87.3
6	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_2\text{H}_4\text{O}\cdot\text{CO}\cdot\text{CH}_3 \\ \text{C}_2\text{H}_4\text{O}\cdot\text{CO}\cdot\text{CH}_3 \end{cases}$	100	86.8	82.2	69.2	79.3	64.1
7	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{N} \begin{cases} \text{C}_2\text{H}_4\text{O}\cdot\text{CO}\cdot\text{C}_3\text{H}_7 \\ \text{C}_2\text{H}_4\text{O}\cdot\text{CO}\cdot\text{C}_3\text{H}_7 \end{cases}$	100	115	93.1	65.7	87.7	87.4
8	$\text{Cl}-\text{C}_6\text{H}_4-\text{SO}_2\text{NHCONHC}_3\text{H}_7$	100	61.0	45.5	55.6	87.3	83.1

$$\text{Hypoglycemic activity} = \frac{\text{sugar contents of treated mouse plasma}}{\text{sugar contents of untreated mouse plasma}} \times 100$$

to an assumption that the same compound may have both the synergistic activity and hypoglycemic activity. Therefore, hypoglycemic activity of sulfonamide derivatives such as Antiresistant and its derivatives was estimated (Table 11) by the method of Somogyi-Nelson as described by Ogita *et al.*<sup>13)</sup> (1965). The experimental results indicated that the excellent synergist for DDT showed an effective hypoglycemic activity to mice. The Antiresistant was the most effective among the chemicals tested. A compound in which butyl group of Antiresistant are substituted with  $-\text{C}_2\text{H}_4\text{Cl}$ , which was a synergist for DDT, showed an effective hypoglycemic activity, whereas substitution with  $-\text{C}_2\text{H}_4\text{OH}$  which brought about no synergistic action failed to show hypoglycemic activity. Hypoglycemic action of the Antiresistant seems to be long acting. Typical results were shown in Fig. 3. Maximum decrease in blood sugar contents of mice occurred 2 hours after the treatment of chlorpropamide, whereas it occurred 8 hours after the treatment of the Antiresistant.

It is still not clear what are the common mechanisms between the synergistic action of

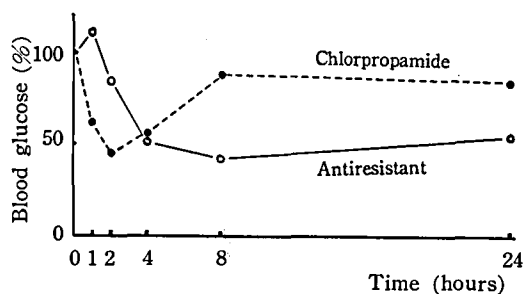


Fig. 3. Blood sugar levels of mice treated with Antiresistant or chlorpropamide. The graph illustrates the more prolonged hypoglycemic effect of Antiresistant over chlorpropamide.

sulfonamides for DDT in house flies and the hypoglycemic action in mice. These two activities are brought about by the same chemical, although they are quite different in character and occur in quite different organisms. Similar relationships were observed in the herbicidal action of sulfonamide derivatives. A sulfonamide, N-diethyl-*p*-chlorobenzene sulfonamide, an ethyl analogue of the Antiresistant, was found to have a considerable herbicidal action to several species of weeds (Matsubara *et al.*, unpublished). Moreover,

relations between chemical structure and the herbicidal action were somewhat parallel to those of synergistic action to DDT. The common mechanisms of a chemical for different actions in different organisms, *e.g.*, synergistic action for DDT in house flies, hypoglycemic action in mice, and herbicidal action in weeds, remains to be clarified.

It has been indicated that the Antiresistant acts as an inhibitor of the enzyme DDT dehydrochlorinase (Wisconsin Alumni Research Foundation<sup>4)</sup> 1961). Kimura and Brown<sup>14)</sup> (1964) showed mosquito DDT dehydrochlorinase was inhibited by DMC and Antiresistant. Tsukamoto and Suzuki<sup>15)</sup> (1964) suggested from their genetic study of DDT-resistance in the house fly that at least two major factors, *i.e.* 5th chromosomal dominant gene and 2nd chromosomal incomplete recessive gene, are responsible for resistance to DDT, and that the gene action of the 5th chromosomal DDT-resistance gene is inhibited by the synergist DMC. It is assumed that the metabolism of DDT to DDE in the house fly is genetically controlled by the 5th chromosomal resistance gene. Oppenoorth<sup>16)</sup> (1964) showed *in vitro* DDE formation in the backcross progeny of his crossing experiments and showed that DDT dehydrochlorinase formation and DDT-resistance were under the control of a single gene on the 5th chromosome. Pillai and Brown<sup>16)</sup> (1965) selected larvae of the yellow fever mosquito, *Aedes aegypti*, with a 1:1 mixture of DDT and Antiresistant, and demonstrated that the mixture-resistance was derived from genetic influences on the 3rd chromosome in addition to the regular DDT-resistance gene on the 2nd chromosome.

As described above, mixture-resistance in the house fly was controlled mainly by 2nd chromosomal recessive gene, indicating the DDT dehydrochlorinase which was produced by the 5th chromosomal DDT-resistance factor is considerably depressed by Antiresistant. Thus it was suggested that the mechanism of synergistic action of Antiresistant is inhibition of DDT-dehydrochlorinase activity in the house fly.

The present genetic study also indicated that there are other factors of DDT-resistance, such as the 2nd chromosomal factor, which is free from dehydrochlorination of DDT. Though Antiresistant

is a useful synergist for DDT, its use for DDT-resistant insects will develop DDT-resistance due to a different factor on the 2nd chromosome from DDT-dehydrochlorination.

### Summary

Biological activities of sulfonamide derivatives were studied. A sulfonamide, N-dibutyl *p*-chlorobenzene sulfonamide, known as WARF Antiresistant for DDT showed marked synergistic action for DDT against moderately DDT-resistant strains of the house fly, whereas it failed to show synergistic action against DDT susceptible strain and highly DDT-resistant strains. Among the sulfonamide derivatives tested *p*-halogenobenzene dialkyl (or monoalkyl) sulfonamides were found to show synergistic activity for DDT. From the structural similarity of synergistic sulfonamides and hypoglycemic sulfonylureas, relations between synergistic action and hypoglycemic action were studied. A chemical which has hypoglycemic activity shows synergistic activity, and *vice versa*.

The genetic analysis of resistance to DDT and to the mixture (DDT+Antiresistant) indicated that DDT-resistance was controlled by two factors, *i.e.* both 2nd and 5th chromosomal ones, whereas the mixture-resistance was controlled by only one factor, *i.e.* the 2nd chromosomal one. It was suggested Antiresistant inhibits the DDT dehydrochlorinase activity which was produced by the gene action of the 5th chromosomal resistance factor. However, it failed to show synergistic action for DDT against some DDT-resistant flies in which resistance was controlled by 2nd chromosomal factor (different from DDT-dehydrochlorinase activity).

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## Genetic Control of Low Nerve Sensitivity to DDT in Insecticide-Resistant Houseflies.

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21. DDT 抵抗性イエバエにおける低神経感受性の遺伝†. 塚本増久\* (大阪大学医学部 遺伝学教室)・植橋敏夫\*\*・山崎雄男 (東京大学農学部 害虫学教室) 40. 10. 31 受理

イエバエの DDT 抵抗性には 2 つの主要遺伝子が関与しており、そのうち第 5 染色体の優性遺伝子は DDT 脱塩酸酵素の活性を支配していることはすでに塚本・鈴木らによって報告されたが、第 2 染色体にある不完全劣性遺伝子の役割については不明であった。一方、山崎・植橋らの研究によって DDT 抵抗性のイエバエは感受性の系統に較べてその DDT に対する神経感受性が低いことが示されている。その後、両者による共同研究の結果、DDT 抵抗性系統のイエバエの低神経感受性は第 2 染色体の不完全劣性遺伝子によって支配されており、神経組織での DDT の解毒とは切り離して考えられねばならないことが明らかとなった。

There are many possible mechanisms of resistance to insecticides in insects, such as reduced penetration of an insecticide into the insect body of sensitive organ, storage of the insecticide in insensitive tissues, enhanced metabolism of the

insecticide, rapid excretion of the insecticide, reduced sensitivity of the nerve to the insecticide, enhanced cholinesterase activity, etc.<sup>2,3,7,10,16,20</sup> Among them, a parallelism between the resistance level to DDT and an increased ability to detoxify

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